

THE VOLATILE TERPENOIDS OF *JUNIPERUS MONTICOLA* f. *MONTICOLA*, f. *COMPACTA*, AND f. *ORIZABENSIS*

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Juniperus monticola, ranging in size from shrub to tree, is widespread in the Neovolcanic Axis across southern Mexico and on peaks of the Sierra Madre Oriental at elevations generally over 3000 m, except for *J. monticola* f. *monticola* at El Chico, Hidalgo, where it occurs at 2450 m (1). Although we have reported on its terpenoid similarities via numerical taxonomic procedures (2), this paper presents the first detailed identification of the oil components. *Juniperus monticola* f. *monticola* is found in the pine-oak communities, whereas both *J. monticola* f. *compacta* and *J. monticola* f. *orizabensis* are found above timberline on volcanic peaks. These sites are quite isolated, and pollen exchange between sites is probably very small. It would appear (1) that the sites of *J. monticola* f. *compacta* and *orizabensis* are more recently available for colonization which suggests that *J. monticola* f. *monticola* would be ancestral with f. *compacta* and f. *orizabensis* being derived species.

MATERIALS AND METHODS

PLANT MATERIALS.—Fresh foliage was collected from *Juniperus monticola* f. *monticola* Mart. at El Chico, Hidalgo, Mexico; from *J. monticola* f. *compacta* Mart. from Nevada de Toluca, Mexico, Mexico; and from *J. monticola* f. *orizabensis* Mart. from Pico de Orizaba, Vera Cruz, Mexico. The collected material was then frozen. Voucher specimens are deposited at the Science Research Center and TEX.

To remove the volatile terpenoids, approximately 200 grams of frozen foliage was subjected to steam distillation for 2 hr (3). The two-hour distillation process removed about 35% of the volatile oil and gives a slight bias toward the more volatile components (3). The oils were kept tightly sealed in vials with foil-lined caps at -20° until analyzed.

Gas chromatographic-mass spectral analyses were run with a Finnigan Quadrupole Gas Chromatograph-Mass Spectrometer, model 4000, (Finnigan Corp, Sunnyvale, California). Mass spectral scans were taken repetitively from mass 41 to mass 300 every second (4).

Chromatographic separation was achieved with a specially deactivated SP 2100 glass capillary column, 0.25 mm ID x 30 meters (J & W Scientific, Supelco Inc.). The column was deactivated by injection of 3 μ l of 50% triethanol amine in methylene chloride (v/v) splitless at 210° and held at that temperature for 2 hrs.

All analyses were made in the split mode (30:1 split ratio) with helium as the carrier with an average linear velocity through the column of 21 cm per sec. The column temperature was held at 55° for 6 minutes after injection and then programmed at 3° per minute to 220° ; 2 μ l of the sample oils were injected after dilution with diethyl ether (1:30). The large amounts of ether were used to achieve better separations via the so-called "solvent effect" (5). Butyl acetate and hexadecyl acetate were added as internal standards. These compounds were chosen as standards because butyl acetate elutes before the most volatile terpenes, and the hexadecyl acetate elutes after most terpenes found in these oils.

Identifications were made by comparison of the ms of each component in the oils with the ms of known terpenes and by searches of spectra from the Finnigan Library of National Bureau of Standards (NBS). Relative retention times (RRT hexadecyl acetate=1.00) were also compared to the RRT of known terpenoids run under the same conditions.

RESULTS

Oils were light to medium yellow with yields from 1–3% dry weight. *Juniperus monticola* f. *monticola* oil is dominated by large amounts of α -pinene and bornyl acetate (table 1), whereas *J. m. f. compacta* is dominated by sabinene and bornyl acetate to a lesser extent, with *J. m. f. orizabensis* having a large amount of bornyl acetate (49%, table 1).

The volatile oils contained 30 components for *monticola*, 43 for *compacta*, and 42 for *orizabensis*. Both *com-*

pacta and *orizabensis* contained several components unique to one species: 10 in *compacta*; 6 in *orizabensis*; but only 1 compound in *monticola* was not found in either of the other three taxa. A total of 52 compounds were found above the trace level, and 33 were common to all three taxa.

The most interesting aspect of these oils is the number of compounds formed only in one or the other of the derived forms (*compacta* and *orizabensis*). Since these two forms occur above timberline where growth rates

TABLE 1. Composition of the volatile leaf oil of *Juniperus monticola* f. *monticola*, *J. monticola* f. *compacta*, and *J. monticola* f. *orizabensis*.

Compound ^{a, b}	% total oil ^c			Compound ^{a, b}	% total oil ^c		
	mont.	comp.	oriz.		mont.	comp.	oriz.
tricyclene.....	0.6	t	0.9	<i>r</i> -terpinene.....	t	3.3	0.6
α -thujene.....	—	0.7	—	(<i>p</i> -menth-1(7),3-diene).....	—	0.5	—
α -pinene.....	25.8	8.8	6.0	C ₁₀ -OH, RRT=0.290.....	—	0.6	—
camphene.....	0.8	t	1.2	unknown, RRT=			
bicyclo (3,2,1)-oct-2-ene, 3-methyl-4-methylene.....	0.5	—	—	0.293.....	t	—	0.9
sabinene.....	(t)	26.9	(t)	terpinolene.....	t	—	0.5
β -pinene.....	0.8	t	t	C ₁₀ -OH, RRT=			
myrcene.....	2.1	2.1	2.8	0.320.....	—	0.9	—
4-carene.....	3.3	0.9	2.1	linalool.....	(t)	t	1.5
α -phellandrene.....	(t)	t	t	isopentyl-isovalerate... (t)	(t)	t	t
3-carene.....	—	—	t	<i>cis</i> -sabinene hydrate... t	t	0.6	1.4
α -terpinene.....	—	1.8	t	camphor.....	3.3	1.0	4.2
<i>p</i> -cymene.....	t	0.5	t	<i>trans</i> -sabinene hydrate... t	t	t	0.7
camphene hydrate.....	0.5	t	1.3	<i>r</i> -cadinene.....	—	t	—
citronellal.....	—	t	—	δ -cadinene.....	—	t	—
borneol.....	4.0	2.5	1.7	elemol.....	2.5	2.3	1.4
4-terpineol.....	t	10.1	0.7	cedrol.....	—	—	t
α -terpineol.....	t	t	t	unknown, RRT=			
citronellol.....	—	t	—	0.733.....	—	—	0.6
piperitone.....	0.9	t	t	<i>r</i> -eudesmol.....	1.0	0.6	t
linalyl acetate.....	—	—	t	β -eudesmol.....	3.3	1.4	t
C ₁₀ -O-Me, RRT=				α -eudesmol.....	1.6	0.5	t
0.474.....	t	t	t	acetate II, RRT=			
bornyl acetate.....	25.6	12.8	48.8	0.862.....	1.4	0.8	t
α -terpinyl acetate.....	t	t	t	manoyloxide.....	t	3.0	t
thymol.....	t	(t)	t	C ₂₀ , RRT=1.017.....	t	1.1	t
caryophyllene.....	—	—	t	manool.....	—	0.6	—
germacrene D.....	—	t	—				
β -phellandrene.....	2.2	0.6	1.5	Total No. of cpds.			
limonene.....	12.4	8.0	13.2	(\geq t).....	36	44	43
<i>trans</i> -ocimene.....	—	—	t	No. of Unique cpds....	1	10	6

^aCompound names in parenthesis are tentatively identified.

^bCompositional values in parenthesis indicate that a compound runs at that retention time but no spectrum was obtained.

^cTrace (t) indicates the compound was less than 0.5% of the total oil. Compounds are listed in order of their retention on SP2100.

are generally quite slow, one might expect rather intense selection for herbivore repellants. There is also ample opportunity for genetic drift in these small, isolated populations. These two factors may play a considerable role in the large changes seen from the ancestral to the derived taxa of this example. It has been shown that different populations of *J. monticola* f. *compacta* can be quite different in their terpenoids (2). It would appear that evolutionary advancement in this case follows an increase in the diversity of the composition of the volatile oil (6-8).

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LITERATURE CITED

1. T. A. Zanoni and R. P. Adams, *Bull. Bot. Soc. Mex.*, **35**, 69 (1975).
2. T. A. Zanoni and R. P. Adams, *Biochem. Syst. Ecol.*, **4**, 147 (1976).
3. R. P. Adams, *Phytochemistry*, **9**, 397 (1970).
4. R. P. Adams, M. Granat, L. R. Hogge and E. von Rudloff, *J. Chromatog. Sci.*, **17**, 75 (1979).
5. K. Grob and K. Grob, Jr., *J. Chromatography*, **94**, 53 (1974).
6. R. P. Adams, E. von Rudloff, T. A. Zanoni and L. R. Hogge, *Biochem. Syst. Ecol.* (in press) (1979).
7. J. W. McClure and R. E. Alston, *Am. J. Bot.*, **53**, 849 (1966).
8. B. L. Turner, *Pure and Applied Chem.*, **14**, 189 (1967).